COMPARATIVE CHEMO-THERAPEUTICAL STUDY OF ATOXYL AND TRYPANOCIDES

PART I

BY

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Atoxyl, sodium-p-amino phenyl-arsenate was introduced in the treatment of Trypanosomiasis by Thomas and Breinl[1] (1905), and its specific therapeutic value for sleeping sickness has been more or less recognised. It contains from 25·95 to 20·78 per cent. of arsenic; the difference depends on the water of crystallisation, as shown by Moore, Nierenstein and Todd,[2] Ehrlich and Bertheim,[3] and others.

Arsenic in the form of Atoxyl is much better tolerated by the animal organism than in the form of sodium arsenate; the therapeutic value of the Atoxyl, therefore, was attributed to the fact that much more arsenic could be administered in this new form. It was supposed to act simply as an internal *antiseptic*, and was thought to kill the parasites in direct proportion to the amount of arsenic introduced.

Some experiments made in June, 1907, by Breinl and Nierenstein seemed to disprove this idea. In an attempt to produce an active immunity against Ngana, mixtures of Atoxyl and trypanosomes were injected in different proportions, and after different periods of contact, with the idea that by increasing the amount of trypanosome-infected blood and decreasing the amount of Atoxyl, and by lessening the time of contact, a point might be reached at which virulent trypanosomes could be injected with impunity.

The results obtained, however, were not what were expected; dogs, rabbits and donkeys were used for the experiments, but invariably after the first injection, even after exposure of the mixture for forty-five minutes to a temperature of 37° C., the animals became infected after a normal incubation period. This fact seemed to suggest that the action of Atoxyl was not simply disinfectant, but was the result of a co-operation between the living tissues and the drug.

Uhlenhuth, Hübner and Woithe[4] in their experimental study of the action of Atoxyl on *T. equiperdum* came to a similar conclusion. They state (p. 296): —

'Unsere Meinung geht jedenfalls dahin, dass der Chemismus der Atoxylwirkung kein so einfacher ist, wie ihn die Theorie der Arsenspaltung supponiert, das vielmehr beim Zustandekommen des wunderbaren therapeutischen Effektes die Körperzelle eine ganz hervorragende Rolle spielt.'

This observation of ours, confirmed by Uhlenhuth, Hübner and Woithe, was the starting point for the following study of the therapeutical action of Atoxyl.

The experiments were divided into two groups, the action of Atoxyl and similar compounds on serum proteids being studied *in vitro* and *in vivo*, respectively. Only the results of the first series are here recorded.

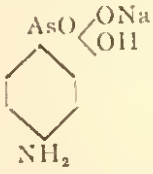
Technique.—20 c.c. of normal serum and 20 c.c. of a 2 per cent. solution of the compound were shaken up for twenty-four hours, and the proteids afterwards were precipitated with 35 c.c. of a 2 per cent. solution of tannic acid. The precipitate was then carefully washed for about forty-eight hours and arsenic estimations of the filtrate were made from time to time until no trace of arsenic could be found in the filtrate. The precipitate was treated with 10-15 c.c. of concentrated sulphuric acid, and digested in a Kjeldahl flask in the usual way.

The arsenic estimations were made by Sanger's[5] method. Instead of hydrochloric acid, gold chloride was used as a developer, and proved much more sensitive.

In those cases in which arsenic was found in the precipitate after digestion, some of the original product was dialysed against water in a parchment sausage-skin, and the dialysate was evaporated to dryness and tested for arsenic.

1. Atoxyl and Serum

Atoxyl =



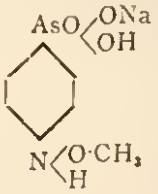
Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Horse serum	arsenic present	—
Horse serum	arsenic present	arsenic absent
Horse serum	arsenic present	arsenic absent
Goat serum	arsenic present	arsenic absent
Goat serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent

2. Sodium Arsenate and Serum

Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Horse serum	arsenic absent	—
Horse serum	arsenic absent	—
Horse serum	arsenic absent	—
Goat serum	arsenic absent	—
Goat serum	arsenic present	arsenic present
Goat serum	arsenic absent	—
Goat serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—
Donkey serum	arsenic absent	—

3. Acetylated Atoxyl and Serum.

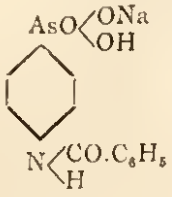
Mono-acetylated atoxyl =



Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent (?)
Donkey serum	arsenic present	arsenic absent

4. Benzoylated Atoxyl and Serum

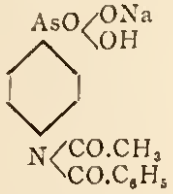
Mono-benzoylated atoxyl =



Serum Used.	Arsenic Estimation in Precipitate.	Arsenic Estimation in Dialysate.
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent
Donkey serum	arsenic present	arsenic absent

5. Benzoyl-acetyl-atoxyl and Serum

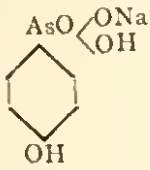
Benzoyl-acetyl-atoxyl =



Serum Used.	Arsenic Estimation in Precipitate.
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent

6. Sodium-p-hydroxyl-phenyl-arsenate* and Serum

Sodium-p-hydroxyl-phenyl-arsenate =



Serum Used.	Arsenic Estimation in Precipitate.
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent
Donkey serum	arsenic absent

It is evident from the foregoing experiments that a combination takes place respectively between proteins and Atoxyl, mono-acetylated Atoxyl, and mono-benzoylated Atoxyl; whilst no combination occurs respectively between these proteins and sodium arsenate, acetyl-benzoyl Atoxyl, and sodium-p-hydroxy-phenyl-arsenate.

It might be mentioned that there is a considerable difference in the results obtained by the treatment of Trypanosomiasis by means of the above-mentioned compounds. Whereas Atoxyl and mono-acetylated Atoxyl act promptly on the parasites, the effect of sodium arsenate is less pronounced, that of sodium-p-hydroxy-phenyl-arsenate is nil.

The analogy between the way in which these compounds behave with proteins, and their action on trypanosomes, is very suggestive. We are, hence, led to believe that this combination with the proteins is of importance in trypanocidal drugs, and have now to consider how Atoxyl and its derivatives become attached to the proteins.

Ehrlich[6] has compared the action of a drug to that of a dye. We know that it is necessary for a dye to possess a chromophoric group—a chemical radical which causes it to be coloured—and a chromogenic

* Our thanks are due to Messrs. Burroughs, Wellcome & Co., who kindly supplied this drug.

group, which renders it a dye. This is easily illustrated by the following example:—

Azo-benzene ($C_6H_5N=NC_6H_5$), which contains the chromophor $N=N$, is coloured, but does not possess dyeing properties. It only becomes a dye when the chromogenic group OH or NH_2 enters. Similarly, for example, oxyazo-benzene ($OH.C_6H_4N=NC_6H_5$) and amino-azobenzene ($H_2N.C_6H_4N=NC_6H_5$) are dyes. Their dyeing value increases with the number of chromogenic groups introduced. For this reason tri-amino-benzene ($NH_2.C_6H_5N=N.C_6H_3(NH_2)_2$) is a much better dye than amino-azo-benzene.

When we apply the same theory to the therapeutics of Atoxyl, we find that sodium-phenyl-arsenate $\left(C_6H_5AsO\begin{matrix}ONa\\OH\end{matrix}\right)$ (which has been proved by Plimmer and Thomson[7], and also in this laboratory, not to possess any curative effect), and also sodium-p-hydroxy-phenyl-arsenate $\left(OH.C_6H_4AsO\begin{matrix}ONa\\OH\end{matrix}\right)$ do not combine with the proteins, whilst atoxyl $\left(NH_2C_6H_4AsO\begin{matrix}ONa\\OH\end{matrix}\right)$ combines with the proteins and acts on trypanosomes; mono-acetylated atoxyl $\left(CH_3CONH.C_6H_5AsO\begin{matrix}ONa\\OH\end{matrix}\right)$ combines and is curative, while fully acetylated and benzoylated atoxyl $\left(\begin{matrix}CH_3CO\\C_6H_5CO\end{matrix}\right)N.C_6H_5AsO\begin{matrix}ONa\\OH\end{matrix}$ does neither.

Hence, we suggest that in Atoxyl the amido group (NH_2-group) and in mono-acetylated Atoxyl the imido group (NH-group) play the same rôle as the chromogenic group in a dye. It has already been pointed out that the action of Atoxyl has generally been explained as being due to the arsenic, and the advantage of its use is that more arsenic could be introduced in the organism in form of Atoxyl than in form of sodium arsenate; it might be argued from this point of view that the action of Atoxyl is as follows:—

The Atoxyl attaches itself to the proteins; the benzene nucleus is slowly oxydised by the tissues and the arsenic is set free; so that,

when combined with the tissues, Atoxyl acts as a storage for effective arsenic.

This, however, is apparently not the case. It is well known that Trypanred, Afridol blue and Afridol violet, also Parafuchsin, have an effect on trypanosomes comparable to that of Atoxyl. These compounds do not contain arsenic, but a large number of amido groups. Further, Laveran,[8] also Thomas and Breinl, have found that sodium arsenate in combination with trypanred acts much better than sodium arsenate alone.

We have, therefore, reason to believe that the amido group in Atoxyl, and in the above-mentioned colouring matters, has a specific action on trypanosomes, and that in Atoxyl the effective part is not only the arsenic, but also the amido group.

How this group acts on the parasites is engaging our attention at present, and will form the subject of a subsequent communication.

LITERATURE

1. Thomas and Breinl. Liverpool School of Tropical Medicine, Memoir XVI, 1906.
2. Moore, Nierenstein and Todd. Bio-Chemical Journal, Vol. II, p. 300, 1907.
3. Ehrlich and Bertheim. Ber. d. deutschen chem. Gesellschaft, Vol. XL, p. 3292, (1907).
4. Uhlenhuth, Hübner and Woithe. Arbeiten aus den Kaiserlichen Gesundheitsamte, p. 256, 1907.
5. Sanger and Black. Jour. Soc. Chem. Inst., Vol. XXVI, p. 1115, 1907.
6. Ehrlich. Deutsche med. Wochenschrift, p. 1052, 1908; also Festschrift für Leyden, Vol. I, p. 645, and Internationale Beiträge zur innern Medizin, Berlin, 1902.
7. Plimmer and Thomson. Proceedings Royal Society. Vol. LXXIX. pp. 505-16 (1907).
8. Laveran. C. R. de l'Acad. des Sciences, Vol. CXL, p. 108, 17 April, 1905.